A simple ^{1}H NMR based assay of total capsaicinoid levels in *Capsicum* using signal suppression in non-deuterated solvent

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Abstract

BACKGROUND: The heat of *Capsicum* fruits are routinely assayed using HPLC to determine capsaicin (CA) and dihydrocapsaicin (DHC) levels. The assay can be time consuming, with each HPLC run typically lasting 10 minutes. Nuclear Magnetic Resonance (NMR) is eminently suitable for quantification of fruit extracts, yet has been largely ignored for quantitative chilli analysis. This study presents a novel approach using solvent suppression in protic solvent (i.e. non-deuterated) to quantify the total level of capsaicinoid in chilli extracts.

RESULTS: Using solvent suppression techniques and maleic acid as an internal standard capsaicinoid content in a series of accurately weighed standard samples was determined over a range between 40 and 720 ppm (0.13 mmolar to 2.35 mmolar) with high accuracy and precision. The measurement was linear over the entire range. This method was subsequently used with ten authentic *Capsicum* samples (seven *chinense*, two *annuum* and one *baccatum*) and showed an excellent correlation with HPLC data. CONCLUSION: The results of the study confirm that NMR in non-deuterated solvent can provide rapid and robust assessment of the pungency of capsicum fruits.

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Keywords

Capsaicinoid quantification; *Capsicum*; chilli fruit; HPLC, NMR analysis

Introduction

Chillies are the fruit of the genus *Capsicum* (family *Solanaceae*). Five species are commonly recognised as domesticated: *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens* and in addition approximately 20 wild species have been reported.[1] Many varieties are widely grown for their fruits which may be eaten fresh, cooked, as a dried powder, in a sauce or processed into oleoresin. Chilli fruits contain many bio-active compounds, including vitamin C and a range of compounds known as carotenoids.[2] However it is the heat of some chillies that attracts most interest.

The main source of heat in peppers is the group of psuedoalkaloid compounds known as capsaicinoids, which are produced in the placenta of the fruit and of which capsaicin and dihydrocapsaicin are by some way the most well represented (Fig. 1). These alkaloids evoke a heat sensation as a result of interactions with ion channels, notably TRPV1 (transient receptor potential cation channels).[3] As an isolated sample capsaicin is a white crystalline solid that is soluble in fats (and organic solvents) but is largely insoluble in water, has no taste and is odourless. Capsaicin has been attributed pharmacological effects since ancient times, however in more recent years extensive research had been done to determine specific applications, including (but not limited to) the gastrointestinal tract,[4] weight-loss[5] and as an analgesic.[6]

Capsaicin and other capsaicinoid levels in *Capsicum* varieties show large differences both in cultivar[7] and as a result of growth conditions.[8, 9] In recent years a series of ever hotter chillies have been grown and reported[10] in pursuit of the crown of 'world's hottest chilli', a title currently claimed by the Carolina Reaper, with a Scoville rating of 2.2 million (SHU – Scoville Heat Unit).

Many techniques for the reliable quantification of capsaicinoid levels in *Capsicum* have been reported. The original method, and indeed the origin of the SHU, was the Scoville Organoleptic Test.[11] This was introduced in 1912 by Wilbur L. Scoville, a pharmacologist, and uses a panel of five people as heat samplers. Exact weights of dried chillies are extracted with alcohol and the resulting solution is serially diluted with sugar water. The panel tastes the solutions until no more heat can be detected in a dilution, with the requirement that three of the five must agree. This method is perforce subjective, and it should be noted that the human palate can quickly become desensitized to capsaicin when testing multiple samples in a short period of time.

More modern methods are now used, including spectrophotometry,[12-15] near-infrared reflectance spectroscopy,[16, 17] thin-layer (paper) chromatography (TLC),[18] gas chromatography (GC),[19, 20] liquid chromatography-mass spectrometry (LC-MS),[21] and high performance liquid chromatography (HPLC).[22, 23] HPLC is now the accepted standard and the American Spice and Trade Association (ASTA) has an agreed method for assaying pungency of chillies. As an analytical technique HPLC has many advantages. It is relatively cheap and easy to run. Standard procedures for analysing chillies have been well established, typically using dried and ground fruit, with organic solvent extraction. Perhaps the biggest limitation for HPLC is the length of run – for full separation of all capsaicinoid components a 20 min run may be appropriate, although detection of capsaicin and dihydrocapsaicin can usually be accomplished with shorter runs.[24]

Nuclear magnetic resonance (NMR) is perhaps the premier analytical technique for organic chemistry but, surprisingly, has not been utilised to any great extent for quantitation in chilli research. Early studies by Kosuge and Furuta used proton (^{1}H) NMR to confirm the identities of isolated capsaicinoids by comparison with samples synthesised from vanillyl amine and authentic fatty acid chlorides.[25] More recently ^{1}H and carbon (^{13}C) NMR have been used for comparative analysis of capsaicinoid purity and structure,[26] and to compare capsaicinoids with capsiate and dihydrocapsiate, novel capsaicinoid esters derived from a cultivar of Capsicum annumm L., CH-19

sweet.[27] Talebi *et al* have employed ^{1}H NMR to allow quantitation of capsaicin in *C. frutescens* where the levels of capsaicin in chilli extracts obtained using microwave assisted extraction were quantified by dissolving a weighed quantity of the dried extract into chloroform-*d* with dimethylformamide (DMF) as an internal standard.[28] No calibration curves were needed as the integration of the signals is assumed to be proportional to the amount of compound, as long as care has been taken in setting up the NMR acquisition (e.g. sufficient signal to noise, complete relaxation of the spin system). Unique peaks are identified in the standard ^{1}H NMR of capsaicin that can be found in chilli extracts and are shown not to overlap with any other signals. H7, for the vanillic CH_2, is chosen, with a 'check' integral of H2, one of the aromatic resonances. If no overlap occurs the integral of H7 should be twice that for H2. It should be noted that no effort is made in the work of Talebi *et al* to discriminate between the different capsaicinoids that may be expected to be present in the chilli extract.

The structures of the capsaicinoids are all closely similar – a vanillylic head unit, an amide link and a long chain alkyl tail (Fig. 1). The first two, the head unit and amide link are common across all the capsaicinoids, only the alkyl chain varies. Typically the variation is also spatially remote from the head unit and amide and as a consequence, the ^{1}H NMR for capsaicinoids is very similar for the aromatic, methoxy and vanillyl methylene signals. Only in the alkyl regions are differences encountered, as illustrated by the ^{1}H NMR spectrum of a mixture of capsaicin (65%) and dihydrocapsaicin (35%). As a consequence use of the H7 protons to quantify capsaicin in chilli extracts will in fact report on all capsaicinoids.

All previous NMR studies of extracted capsaicinoids have involved removal of the original solvent used for extraction.[28, 29] Primarily this is due to the requirement for deuterated solvents for NMR, and the need to remove potentially large solvent signals that could obscure relevant peaks in the resulting spectrum. However recent advances in NMR technology, notably gradient shimming[30, 31] and methods for solvent suppression[32] have allowed NMR to be contemplated in proteo-solvent and without the need for deuterium. In this study the capsaicinoid levels of ten chilli cultivars (*C.*

chinense, C. annuum and *C. baccatum*) were determined using HPLC and ^{1}H NMR. Crucially the ^{1}H NMR assay is conducted *using the same solution as the HPLC* – no further preparation is required. By obviating the need for solvent removal and by using proteo-solvent to measure the NMR spectra significant savings in time (solvent removal) and money (cost of deuterated solvent) can be made.

Experimental

Plant Material

The chilli fruit were obtained as a gift from Simpsons Seeds, The Walled Garden Nursery, Horningsham, Wiltshire, BA12 7NQ. UK. Fruit were collected ripe from the plants and were stored in a freezer prior to use.

Reagents

HPLC grade acetonitrile (VWR International) was used. Water was purified by reverse osmosis, and then filtered. The mobile phase (a binary mixture of acetonitrile and water at 65:35 ratio) was degassed *via* ultrasound for 5 min prior to use. Chloroform-*d* (99.8 atom % d) and maleic acid (Standard for quantitative NMR) and were obtained from Sigma-Aldrich and used without further purification. Capsaicin and dihydrocapsaicin were obtained from Sigma-Aldrich as a stated mixture of capsaicin (65%) and dihydrocapsaicin (35%). This ratio was more accurately determined by ^{1}H NMR analysis of 1 mg in chloroform-*d* and the ratio was found to be capsaicin (68.3%) and dihydrocapsaicin (31.7%) by integration of the alkyl chain methyl signals for the two compounds (for capsaicin the doublet is at 0.95 ppm, for dihydrocapsaicin 0.85 ppm) and this ratio was used for subsequent calculations.

Method development

A stock solution (0.212 mmolar) was prepared from maleic acid (24.6 mg) in acetonitrile (25 mL), equivalent to 0.984 mg mL^{-1}. Six solutions of capsaicin/dihydrocapsaicin in acetonitrile were prepared, each with 2.5 mL of the maleic acid stock solution, giving a maleic acid concentration of

0.0984 mg mL^{-1}. The final concentrations were 0.491, 0.273, 0.191, 0.136, 0.068 and 0.027 mg mL^{-1} for capsaicin and 0.228, 0.127, 0.089, 0.063, 0.032 and 0.013 mg mL^{-1} for dihydrocapsaicin.

^{1}H NMR analyses were performed using a Bruker Avance III NMR spectrometer operating at 500.13 MHz (for ^{1}H). The acetonitrile solutions were used directly, no deuterated solvent was used. Samples were run in unlocked mode, as no deuterium lock signal was present and shimming was achieved using the large solvent signal in ^{1}H mode with the Topshim (Bruker) routine. ^{1}H spectra were obtained using a presaturation[33] solvent suppression pulse sequence (noesygppr) to reduce the intensity of the acetonitrile peak. Typical experimental conditions are: 16 transients were obtained at 298 K, an acquisition time of 1.586 s, a recycle delay of 20 s. The spectral width was 10,330 Hz (~20 ppm). The acquired points were 16384, with zero filling to 65536 points.

In order to ensure that the samples had fully relaxed between transients a series of spectra were acquired on the most concentrated sample with a varying relaxation delay (d1). This established that a d1 of 20 s was sufficient. Quantitation was achieved by comparison of the integral for the two maleic acid protons at 6.20 ppm with the integral for the aromatic peak at 6.84 ppm (this represents one of the three aromatic head group protons). In order to calculate the weight of capsaicinoid present an average M_w was chosen to reflect the small differences between the various compounds and their usual distribution profile taken from the HPLC data. In this study the average capsaicin:dihydrocapsaicin ratio was 2.15:1, and thus an estimated average Mw for capsaicinoids of 306.06 g mol^{-1} was used. Each solution was measured by ^{1}H NMR three times. In order to ensure reproducibility several samples were also analysed on other NMR instruments at the University of Bath (a 400 MHz Bruker Avance III, and a 500 MHz Agilent Propulse).

Capsaicinoid Extraction

For analysis several fruit of each cultivar were sliced open and then dried in an oven at 60 °C for 48-72 hours.[24] The chilli samples were ground to a fine powder using a standard culinary spice grinder. Weighed amounts of dried chilli were extracted using acetonitrile at 40-50 °C in an

ultrasound bath for 30 minutes.[22] Typically 0.5 g of chilli powder was extracted with 40 mL of acetonitrile. Following extraction the solutions were filtered (cellulose filter paper, Whatman) and made to an accurate, known volume (50 mL). For each cultivar three separate extractions were performed.

HPLC Analyses

The HPLC analyses were performed by using a system consisting of a sample injector, a JASCO PV-1580 pump, a JASCO UV-1575 detector and a BDS Hypersil C18 column (150 x 4.6 mm) with a flow rate of 1 mL min^{-1}. The column temperature was at 25°C and the wavelength employed for the detection was 222 nm. The mobile phase consisted of a binary mixture of acetonitrile and water at 65:35 ratio. The run time for each chilli sample was 10 min and 20 µL of the sample was injected into the column in each analysis.

The HPLC response was calibrated as follows. A stock solution of the capsaicin standard was prepared by dissolving 50.0 mg of the standard (containing 34.54 mg capsaicin, 15.46 mg dihydrocapsaicin, using the ratio determined by ^{1}H NMR above) in 100 mL of acetonitrile, giving concentrations of 0.345 mg mL^{-1} (capsaicin) and 0.155 mg mL^{-1} (dihydrocapsaicin). Five further dilutions (in acetonitrile) were also used to afford standards with concentrations of 0.173, 0.086, 0.043, 0.034 and 0.007 mg mL^{-1} (capsaicin) and 0.077, 0.039, 0.019, 0.016 and 0.003 mg mL^{-1} (dihydrocapsaicin) respectively. Each standard was assayed to give three consistent chromatograms. The average figure for area was used and a calibration line of best fit was generated. Both calibrations gave a linear response for the range of concentrations under study (R^2 for capsaicin was 0.9997, for dihydrocapsaicin it was 0.9996).

Each of the three extracts for the ten cultivars was assayed under the same conditions. Three repeated injections for each sample were typically within 1 to 2%. The peaks for capsaicin and dihydrocapsaicin were well resolved, with retention times of 3.34 and 4.10 min respectively. To

ensure consistency the standard containing capsaicin (0.173 mg mL^{-1}) and dihydrocapsaicin (0.077 mg mL^{-1}) was injected every ten samples with no variation seen in the observed chromatograms.

NMR Analyses

Each of the 30 samples was investigated by ^{1}H NMR using the method described above.

Results and discussion

The use of deuterated solvents in NMR generally serves two purposes. Firstly by replacing the vast majority of solvent protons with deuterium, the potential problem of a very large solvent peak or peaks is removed. Secondly the presence of a deuterium signal allows the sample to be 'locked', whereby measurement of the ^{2}H NMR allows compensation for any drift of the magnet to be achieved. If a fully proteo solvent is to be employed for NMR analysis these problems need to be addressed. The need for locking the sample is negligible as the anticipated acquisition times are sufficiently short (minutes) that no significant drift would be encountered. The large solvent peak can be suppressed by any of a number of techniques developed over the years for biological samples that are typically measured in 90 % H_2O, 10 % $^{2}H_2O$ or more recently in conjunction with LC-NMR techniques.

The use of maleic acid as a standard for quantitative NMR is well established.[34] It has only a single resonance, which is preferable to avoid overlap with analyte or solvent signals. It is an easily weighed, stable solid and is available in very high purity. In order for quantitation by NMR to be valid care must be taken with acquisition parameters, notably to ensure that the spins have fully relaxed between pulses. For capsaicin, and indeed chilli extract samples in general, there exists a window in the ^{1}H NMR spectrum between 5.4 and 6.6 ppm – maleic acid in acetonitrile gives a singlet at 6.33 ppm (Fig. 2).

In order to establish if ^{1}H NMR using solvent suppression, combined with maleic acid as an internal standard, could be used to accurately quantify the levels of capsaicin/dihydrocapsaicin in acetonitrile extracts, method development was undertaken. Initially six solutions of varying concentrations of capsaicin/dihydrocapsaicin were prepared, each with the same concentration of maleic acid. In order to ensure that all the spins were fully relaxed between pulses a series of acquisitions were undertaken with a varied relaxation delay (d1). As can be seen in Fig. 3 a delay of 20 s was sufficient to ensure that full relaxation occurred and that the integrations of the signals was valid.

Using these parameters the six solutions were each analysed three times, on discrete samples and over a number of days. As can be seen in Table 1 the experimentally derived concentrations, based on the integration of the maleic acid signal against that of the capsaicinoid aromatic signal are in close agreement, and a linear regression of a plot of NMR derived concentration vs actual concentration has R^2 = 0.9997 (see supporting information for full details). Precision reflects the consistency of a series of measurements in regard to obtaining the same value on multiple occasions, and in this case the coefficient of variation has been used, where the Coefficient of Variation = (Standard Deviation/Mean) x 100. The coefficient of variation is small for all the samples, with the largest spread observed for the most dilute sample. This is not surprising as at this concentration the influence of relatively small signal-to-noise artefacts will be most prevalent. However the signal-to-noise for this sample is still reasonable – 14.7 (calculated using the region 7.72 to 8.26 ppm for baseline and 7.50 to 7.60 ppm for signal).

The % Accuracy, also described as the % Recovery, is calculated by diving the measured value by the true value and expressing as a percentage. In this case the % Accuracy is consistently within 5 % of the true values for all the concentrations investigated.

In order to determine in the linear range of the method a plot of the standardised residuals against the expected concentration was used (Fig. 4). No systematic pattern was observed through

visual examination and none of the standardized residuals exceeded the 95 % confidence limit (± 2 standard deviations), suggesting that the response is linear across the range under study.

In order to test for reproducibility the samples were analysed on two further NMR spectrometers, one an Agilent ProPulse 500 MHz spectrometer and a Bruker 400 MHz Avance III spectrometer. No significant differences were observed across the three spectrometers (see supporting information). As expected the samples recorded at the lower magnetic field strength give smaller signal to noise ratios, and the ability to fully suppress the solvent signal is reduced. The latter effect is primarily attributed to the spectral dispersion at the lower field. However all the samples investigated at 400 MHz gave consistent results with those obtained at a higher field strength.

The use of Fourier Transformation in NMR allows for multiple scans (transients) of a sample to be taken, and combined in order to improve sensitivity. Owing to the nature of the process the signal-to-noise increases at the rate of $\sqrt{2}$ x ns (ns = number of scans). Using the 500 MHz Avance III spectrometer the signal-to-noise on the lowest concentration sample was 15:1 and for the highest 275:1, based on 16 transients and an acquisition time of about 6 minutes. A signal-to-noise ratio between 3 and 2:1 is generally considered acceptable for estimation of limit of detection (LOD), and this suggests that for the lowest concentration chosen here this consideration is met. For limit of quantitation (LOQ) a typical signal-to-noise ratio of 10:1 is typically accepted for reliable accuracy and precision. For the more concentrated samples 16 scans would clearly not be required to satisfy this, and thus depending on concentration of the samples under study, this method should allow accurate capsaicinoid determination in less than 5 minutes.

Following the thorough validation of the method on artificial solutions, a series of chilli samples were assayed using the novel NMR method and for comparison using the more accepted standard HPLC approach. Ten authentic *Capsicum* samples (seven *chinense*, two *annuum* and one *baccatum*) were chosen to give a range of both cultivar and heat (capsaicinoid level). For each sample several ripe fruit were dried over several days at 60 °C and then ground to a fine powder

using a standard culinary spice grinder. Three separate samples were generated for each cultivar, and each were extracted with acetonitrile in a warm ultrasound bath for 30 minutes. Each solution was filtered and made up to an accurate volume with a consistent loading of maleic acid as internal standard. The NMR spectra and HPLC data were acquired in triplicate (see supporting information for full details). As can be seen in Fig. 5 the two methods correlate extremely closely, with an R^2 of 0.997.

Collins *et al* reported that using less than 1 g of sample results in statistically significant differences in measured capsaicinoid levels.[24] In the present study smaller samples were used, however this is reasonable as the intention was not quantification of capsaicinoid levels *per se*, rather that the novel NMR method would give equivalent results to those determined by HPLC, and this is the case. It is interesting to note that while there is variation in the capsaicinoid levels determined for each sample, the differences are not statistically significant (unshown results).

As shown in Fig. 2 capsaicin and dihydrocapsaicin can be differentiated in the ^{1}H NMR spectrum by using the integration of the terminal methyl signals at 0.95 ppm (capsaicin) and 0.85 ppm (dihydrocapsaicin). This approach is not viable for actual chilli samples owing to the presence of further peaks in the spectrum that substantially overlap these signals (Fig. 6). The extra peaks arise from minor capsaicinoids, and in addition from the presence of the terminal methyl triplets from mono-, di- and triglycerides.

This inability to discriminate between individual capsaicinoids is a potential limitation of using NMR for heat quantification. Whilst capsaicin and dihydrocapsaicin routinely comprise the greatest fraction (up to 90 %), minor capsaicinoids are always present, and indeed for low heat chillies may well feature to a much greater relative proportion (unpublished results). Short run HPLC experiments tend not to separate such components fully, so that a peak described as capsaicin may include nordihydrocapsaicin, and the second main peak, dihydrocapsaicin, may also include homodihydrocapsaicin and potentially n-vanillyl-n-decamide.[24] Longer run HPLC clearly separates

these peaks. In order to report the levels of capsaicinoid in a given sample a typical measure is the parts per million of heat (ppmH), and this can be converted into a Scoville Heat Unit (ppmH x 15)[24]. Capsaicin and dihydrocapsaicin are usually attributed 16,000,000 and 15,000,000 SHU respectively, although it is unclear as to the ultimate source of these numbers, and numbers for other capsaicinoids are regularly encountered in popular literature without specific attribution. In addition the heat induced by individual capsaicinoids varies, so that nordihydrocapsaicin has been described as the 'least irritating' with a 'mellow warming effect', while capsaicin and dihydrocapsaicin elicit a more typical heat sensation.[19, 35] It is also known that perception of heat for chillies does not always agree with analytical methods, so that *C. pubescence* is frequently cited as hotter than *C. annuum*, which is reported to have higher parts-per-million capsaicinoid concentration. With the widespread acceptance of the ppmH and SHU as measures of chilli heat, the facile measurement of the total capsaicinoid content described herein should be seen as a counterpoint, and may provide useful further information.

Conclusions

^{1}H NMR in protic solvent clearly provides a highly accurate and rapid method for measuring the total capsaicinoid content of chilli samples. The method described here has been validated for accuracy, precision, linearity, repeatability, and robustness. The NMR equipment used is now standard in most synthetic chemistry environments, and the experimental methods can be implemented very easily by any trained NMR spectroscopist. The method yields a rapid determination of the total capsaicinoid content, with a routine acquisition time of 1-2 minutes being typical, compared to longer for HPLC. In addition, the use of protic solvent removes the requirement for sample manipulation prior to generation of the NMR sample and also vastly reduces the cost compared with using deuterated solvents. While the method does not provide information on the amounts of different capsaicinoids present, and thus a direct conversion to either ppmH or SHU is

not possible, the authors suggest that the capsaicinoid content determined in this way provides additional information to more traditional assessments of heat.

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Data Access Statement

All NMR spectra and HPLC traces are available from the author on request.

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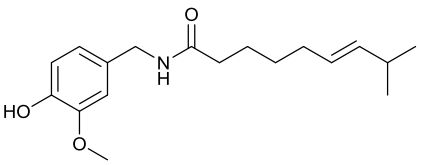
Table 1 Experimentally derived concentrations for the standard capsaicinoid samples, including details of Precision (measured by Coefficient of Variation) and % Accuracy. [†]Concentration determined by comparing integral of maleic acid protons as internal standard against aromatic head unit protons for capsaicinoids. Quoted concentration is mean (n = 3). [‡]Coefficient of Variation = (Standard Deviation/Mean) x 100. [§]% Accuracy is calculated by diving the measured value by the true value and expressing as a percentage.

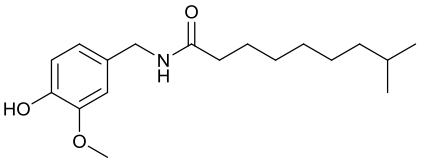
Concentration / mg mL^{-1}	NMR concentration[†]/ mg mL-1	Std. dev.	Coefficient of variation (%)[‡]	% Accuracy[§]
0.718	0.718	0.00370	0.52	100.0
0.400	0.392	0.00297	0.75	98.4
0.280	0.280	0.00188	0.57	100.1
0.199	0.194	0.00196	1.01	97.3
0.100	0.097	0.00214	2.19	97.7
0.040	0.041	0.00220	5.42	101.5

Illustrations

Figure 1 Structures of (E)-N-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide (capsaicin, a) and (E)-N-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide (dihydrocapsaicin, b).



Capsaicin (**a**)



Dihydrocapsaicin (**b**)

Figure 2 Example ^{1}H NMR spectra showing capsaicin, dihydrocapsaicin and maleic acid signals, recorded in proteo-acetonitrile, unlocked and with solvent suppression of the acetonitrile signal at 1.96 ppm. Legend is as follows (a) aromatic signal from capsaicinoids used for integration, (b) maleic acid, (c) capsaicin methyl signal, (d) dihydrocapsaicin methyl signal, (e) suppressed acetonitrile signal, showing ^{1}H-^{13}C satellite peaks.

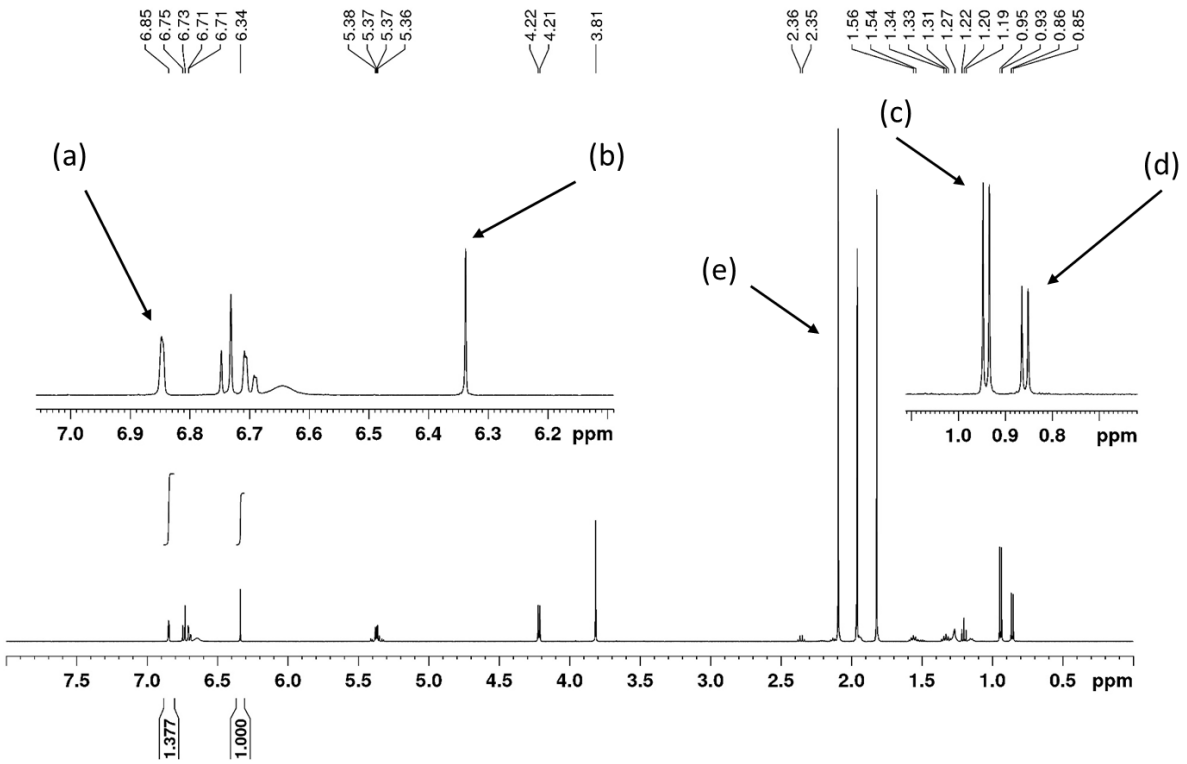


Figure 3 Experimentally derived concentration of capsaicin/dihydrocapsaicin against relaxation delay (d1). The experimental value reaches the 'true' value at 20 s.

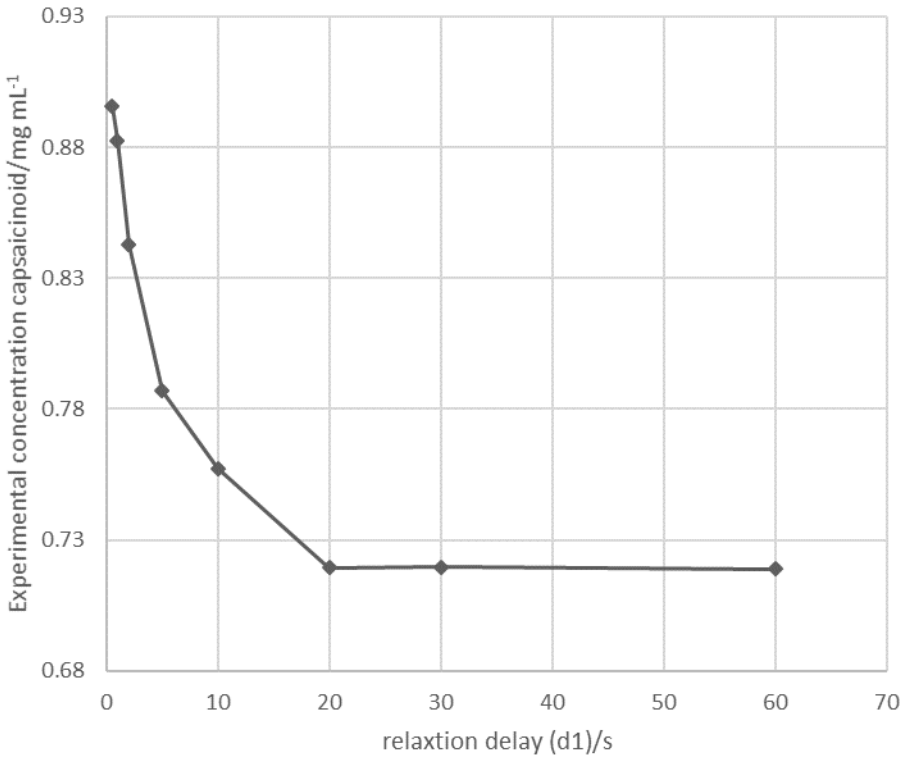


Figure 4 Plot of standardized residuals against concentration, showing no systematic pattern across the linear range and all residuals remaining within ± 1.96 (95 % confidence of linearity). Dotted lines represent the ± 1.96 limits.

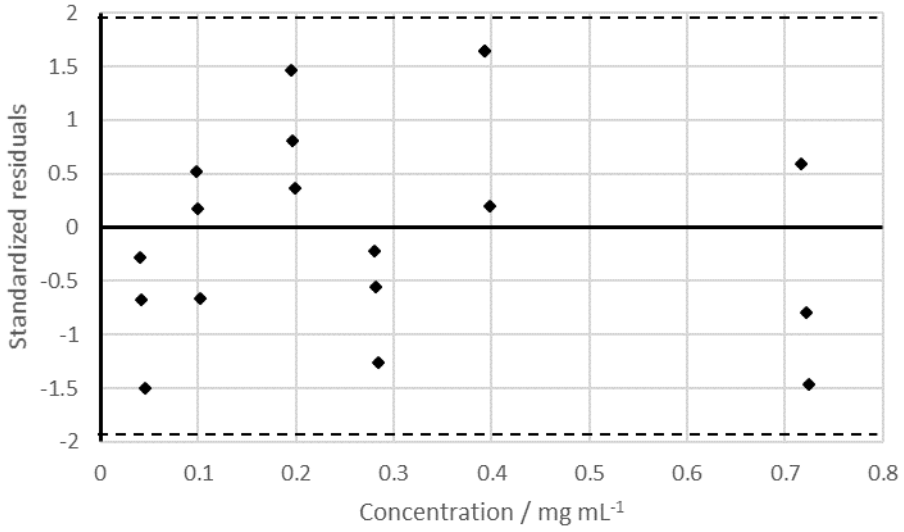


Figure 5 Plot of capsaicinoid concentration determined by ^{1}H NMR against concentration determined by HPLC for ten different chilli cultivars. Each cultivar was assayed three times, and the determinations are the mean value (n = 3) for both NMR and HPLC.

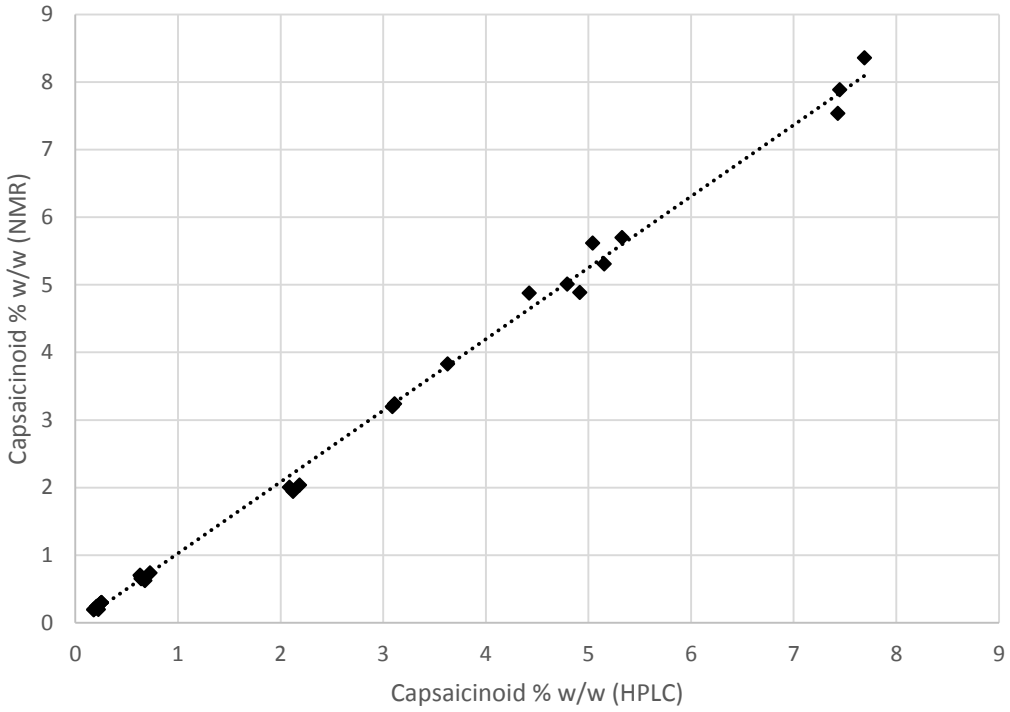


Figure 5.

Figure 6 Comparison of the methyl signals in the ^{1}H spectrum of chilli samples, showing the presence of overlapping peaks which preclude direct integration. The spectra are as follows (a) capsaicin/dihydrocapsaicin standard, (b) *C. chinense* 'Paper lantern', (c) *C. chinense* 'Dorset naga' and (d) *C. chinense* 'Moruga Scorpion.

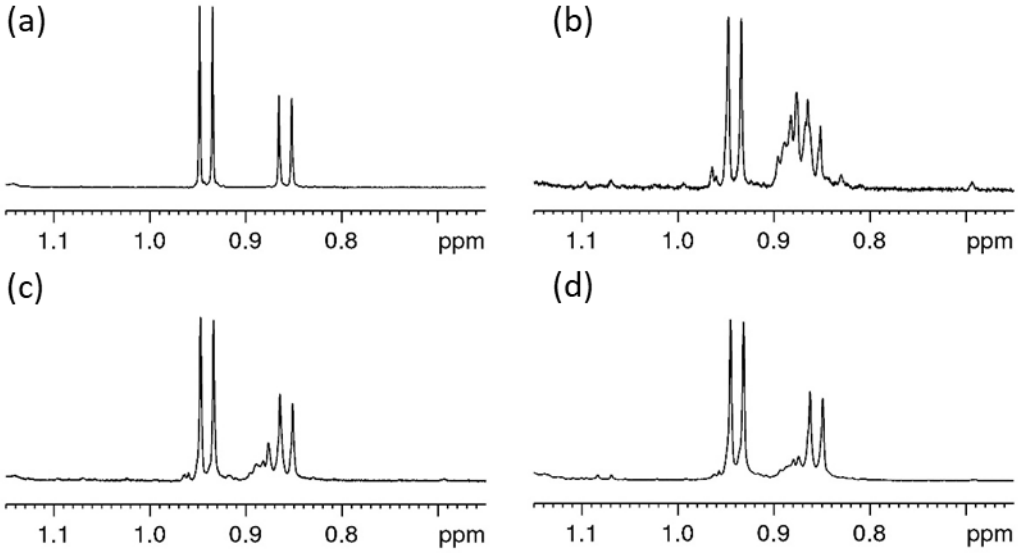


Figure 6.